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The Precision of the ASTM Bioconcentration Test

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THE PRECISION OF THE ASTM BIOCONCENTRATION TEST

Prepared for

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Prepared by

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Duluth MN 55804

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Superior, WI 54880

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16. ABSTRACT

The ASTM method for measuring the bioconcentration factor (BCF) of chemicals was evaluated using 1,2,4-trichlorobenzene (TCB), hexachlorobenzene (HCB), and p,p'-DDE (DDE). Four replicate, 28-day exposures of the chemicals to fathead minnows were used to determine the precision of the test method. Using the 28-day values, the mean (±S.D.) BCF for TCB, HCB, and DDE were 1,700 (±70), 35,000 (±3,300), and 50,000 (±4,800), respectively. The results showed that steady-state residues are not attained for highly bioaccumulative chemicals in the 28-day exposure, and the calculation of the BCF by dividing the 28 day residues by the mean water concentration is inadequate. Two alternate methods of calculating the BCF are discussed.

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The Precision of the ASTM Bioconcentration Test

by

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INTRODUCTION

The purpose of this study was to evaluate the bioconcentration factor (BCF) X test method suggested by ASTM (1) through the participation in an interlaboratory round-robin testing program. Although an evaluation of the round-robin tests will be published elsewhere, this laboratory examined the precision of the method in four replicate tests using three chemicals as well as several different methods of estimating the bioconcentration factor from the exposure data. Hexachlorobenzene (HCB), p,p'-DDE (DDE) and 1,2,4-trichlorobenzene (TCB) were selected for the round-robin evaluation because they were anticipated to exhibit varying depuration rate constants and bioconcentration factors while minimizing complications of metabolism and of the need for using dissimilar analytical methods.

Discussions of the bioconcentration process have been published (2, 3, 4, 5). Bioconcentration is defined as the direct uptake of a chemical into aquatic organisms through the gill or other membranes. The bioconcentration factor is the ratio of the chemical residue in the fish tissue and the concentration of the chemical in the water after a steady-state is observed. Branson et al. (2) proposed that the uptake process can be modeled by the first order relationship:

$$\frac{dC_F}{dt} = K_1 C_w - K_2 C_F$$

where C_w and C_F are the chemical concentrations in the water and fish, respectively, and K_1 and K_2 are the uptake and depuration rate constants, respectively. Since steady-state is defined as the point where $dC/dt = 0$, it is clear that chemicals which have small depuration rate constants, i.e. $K_2 \rightarrow 0$, will require long exposure times in order to observe steady-state.

Consequently, one method to estimate the BCF using the ratio of C_F/C_w at the end of an arbitrary exposure period may only be an accurate measure of the bioconcentration factor for chemicals with appreciable depuration rates where steady-state is reached quickly. For chemicals which are not depurated rapidly the ratio may underestimate the steady-state bioconcentration factor because the residues continue to increase throughout the exposure.

Branson et al. (2) proposed that this problem can be alleviated by defining the bioconcentration factor as the ratio of the rate constants, K_1/K_2. This eliminates the need to expose fish until steady-state is achieved, but it introduces the uncertainity of extrapolating beyond the exposure data and the tendency to amplify variability in the analytical measurements by dividing by a small number. The computer program for this model provided by Dow Chemical called BIOFAC was used as a second method to estimate the bioconcentration factor in this study.

A third method used to estimate the bioconcentration factor was similar to the BIOFAC in that it assumed the uptake was a first order rate process. Integrating the uptake equation gives $C_F = (K_1/K_2)C_w(1-e^{-k_2t})$. If $BCF \simeq K_1/K_2$, then $C_F/C_w = BCF(1-e^{-k_2t})$. This is similar to the equation proposed by Ernst (3). Therefore, if the values of C_F/C_w are measured for varying time periods, t, a non-linear least squares analysis can be used to fit the data and estimate the steady-state BCF. This least squares analysis of the data was compared to the other methods of estimating the bioconcentration factor.

EXPERIMENTAL PROCEDURES

Exposure System

The test system consisted of a syringe injector exposure system described by DeFoe (6). The three chemicals were tested simultaneously in quadruplicate by preparing acetone stock solutions containing appropriate amounts of HCB, DDE, and TCB to produce exposure concentrations of approximately 0.2, 0.15, and 20 µg/l, respectively, when 8 µl of acetone was injected into each liter of dilution water. The control tanks received an equal quantity of acetone.

The test water was unfiltered Lake Superior water heated to 21°C ± 0.5°C and contained greater than 90% DO saturation. Hardness and pH measured at the initiation of the test were 45-47 mg/l (as $CaCO_3$) and 7.8, respectively. Other chemical characteristics of Lake Superior water may be found in Biesinger and Christensen (7).

The test organism used in the bioconcentration test was the fathead minnow (Pimephales promelas) obtained from the Environmental Research Laboratory-Duluth culture. Juvenile fish weighing 0.1-0.15 g were fed a daily diet of frozen brine shrimp (San Francisco Bay Brand) supplimented with dry trout chow pellets (Glenoce Mills). The trout chow was purchased for experimental work and was previously shown to be free of significant quantities of pesticides. Handling, holding and acclimation procedures for the fish were followed according to the ASTM guidelines for conducting bioconcentration tests (1).

After chemical analyses verified stable exposure concentrations in the test chambers, 40 fish were transferred to each tank containing 27 liters of water. The flow rate of incoming water was 250 ml per minute. Samples of 4 fish from each tank were randomly removed on day 0, 2, 4, 8, 16, and 28 of

the uptake phase and on day 35, 49, and 56 of the depuration phase. The fish were removed from the tanks and placed in a beaker of ice water. After all movement ceased, they were blotted dry, weighed, and frozen in solvent-rinsed glass vials.

The water samples were siphoned directly from the tank into a 500 ml volumetric flask (250 ml for control tanks) to which 25 ml of hexane had previously been added. A 1.5 inch teflon coated stirring bar was placed in the flask and the sample was vortex mixed for 1 hr. The two phases were allowed to separate for 0.5 hr, and a 1.5 ml aliquot of hexane was transferred to a gas chromotography injection vial for analysis.

The accuracy of the analytical methods was checked by determining the percent recovery of known amounts of 1,2,4-trichlorobenzene, hexachlorobenzene, and p,p'-DDE in the water. The percent recovery for the water analysis was 95.4%, 98.6%, and 102.8%, for TCB, HCB, and DDE, respectively (N=6). The water concentrations were corrected for percent recovery.

Analytical Methods

Composite whole fish samples were homogenized with 40 gm of granular anhydrous Na_2SO_4 (Mallinckrodt Inc.). The powdered homogenate was transferred to a 300 ml chromatographic column and eluted with 250 ml of redistilled hexane into a 250 ml volumetric flask. Because of the high concentration, no cleanup procedure was performed. After the necessary dilutions were made, quantitation of TCB, HCB, and DDE was performed by gas chromatography.

The lipid content of each tissue sample was determined gravimetrically using a drying period of 15 minutes at 110°C. The lipid content ranged from

6.1% to 9.8% with a mean value of 8.44 $\pm$ 0.8 with N=23.

The gas chromatographic analyses were performed on a 5730A Hewlett-Packard gas chromatograph with an auto sampler and a Hewlett-Packard 3354B lab automation data system. The gas chromatograph was equipped with a ^{63}Ni electron capture detector held at 300°C. The injection port temperature was 250°C. The 6 ft 2 m x 3 mm (OD) glass column was packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh supelcoport (Supelco Inc.). The carrier gas was 5% methane in argon with a flow rate of 40 ml per minute.

The water samples were analyzed at a programmed column temperature of 140°C to 190°C at 4°C per minute. The tissue samples were anlayzed at a programmed column temperature of 100° to 220°C at 8°C per minute for TCB and HCB and at 200°C isothermal for DDE. The percent recovery for spiked tissue samples were 102% with N=3 for TCB 99% with N=3 for HCB and 111% with N=3 for DDE. The tissue concentrations were corrected for recovery.

RESULTS

The results of the bioconcentration tests using TCB are presented in Table 1. The data show that the water concentrations decreased approximately 20% from the initial concentrations during the 28 day exposures. TCB was not detectable in the initial test fish but had accumulated to 15.8-18.4 µg/g after two days of exposure. After the initial rapid uptake, the concentrations increased slowly to maximum concentrations of 19.0-26.3 µg/g in the four exposures. The ratio of C_F/C_w for TCB in Exposure 1 ranged from 1,080 after two days to 1,640 after 28 days. The C_F/C_w in Exposure 2 ranged from 1,170 after two days to 1,770 after 28 days. The C_F/C_w in Exposure 3 ranged from 1,070 after two days to 1,800 after 28 days. The C_F/C_w in Exposure 4 ranged from 1,070 after 2 days to 1,730 after 28 days. TCB was eliminated rapidly from fish during the depuration phase of the study. All fish contained 0.60 µg/g or less seven days after the chemical ceased to be added. After 21 days in clean water the fish contained approximately 0.1 µg/g.

The results of the bioconcentration tests using HCB are presented in Table 2. The water concentration decreased approximatley 15% during the exposure and the mean concentrations were all approximately 0.15 µg/1. HCB concentrations in the test fish were initially about 0.09 µg/g. After two days of exposure, the HCB concentration increased ten-fold to approximately 1 µg/g. In contrast to the TCB exposures, HCB residues continually increased during the 28-day tests to maximum concentrations ranging from 4.55 to 6.22 µg/g in the four tests. The C_F/C_w in Exposure 1 ranged from 5,700 after two days to 33,000 after 28 days. The C_F/C_w in Exposure 2 ranged from 7,400 after two days to 32,000 after 28 days. The ranges of two day and 28 day C_F/C_w in Exposures 3 and 4 were 5,700 to 39,000 and 5,400 to 37,000,

TABLE 1. Summary of Analytical Results from 1,2,4-Trichlorobenzene Bioconcentration Tests

Test Day	Exposure 1				Exposure 2				Exposure 3				Exposure 4			
	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_F}{C_w}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_F}{C_w}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_F}{C_w}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_F}{C_w}$
0	14.6[a]	<0.01[b]	6.06[c]	-	13.8	<0.01	6.06	-	17.1	<0.01	6.06	-	17.2	<0.01	6.06	-
2	14.6	15.8[d]	7.99	1,080	13.8	16.1	7.20	1,170	17.1	18.3	7.91	1,070	17.2	18.4	8.02	1,070
4	13.9	15.4	7.55	1,100	13.7	19.5	8.27	1,420	16.1	21.6	8.81	1,340	17.6	21.8	8.59	1,240
8	13.3	16.1	NA	1,200	13.5	17.6	7.92	1,300	15.1	22.9	8.70	1,520	17.0	19.4	8.01	1,140
16	12.3	19.0	9.28	1,540	12.9	16.9	8.69	1,310	14.1	22.2	8.94	1,570	16.4	22.2	9.00	1,360
28	11.3	18.5	9.22	1,640	12.4	21.9	9.52	1,770	13.4	24.1	8.52	1,800	15.2	26.3	9.27	1,730
35	0.1	0.25	8.77	-	0.1	0.41	9.23	-	0.1	0.44	8.54	-	0.2	0.60	9.67	-
49	<0.1	NA	6.63	-	<0.01	0.05	7.77	-	<0.01	0.11	7.71	-	<0.01	0.11	8.45	-
56	<0.1	0.03	5.82	-	<0.01	0.04	8.24	-	<0.01	0.06	6.32	-	<0.01	0.16	6.85	-

[a] Water concentrations expressed as mean of all analyses prior to fish sampling day.

[b] Initial fish concentration estimated from mean of control fish analyses during depuration phase.

[c] Lipid content of composite fish samples.

[d] Fish tissue analyses of composite fish analyses.

NA = not analyzed.

TABLE 2. Summary of Analytical Results from Hexachlorobenzene Bioconcentration Tests

Test Day	Exposure 1				Exposure 2				Exposure 3				Exposure 4			
	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_F}{C_W}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_F}{C_W}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_F}{C_W}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_F}{C_W}$
0	0.19	0.09	6.06	-	0.15	0.09	6.06	-	0.16	0.09	6.06	-	0.17	0.09	6.06	-
2	0.19	1.08	7.99	5,700	0.15	1.10	7.20	7,400	0.16	0.92	7.91	5,700	0.17	0.93	8.02	5,400
4	0.18	1.53	7.55	8,500	0.16	1.63	8.27	10,000	0.17	1.61	8.81	9,400	0.18	1.54	8.59	8,600
8	0.17	2.23	NA	13,000	0.17	2.49	7.92	15,000	0.15	2.75	8.70	18,000	0.18	2.53	8.01	14,000
16	0.15	3.74	9.28	25,000	0.16	3.72	8.69	23,000	0.14	4.33	8.94	31,000	0.18	3.97	9.00	22,000
28	0.14	4.55	9.22	33,000	0.16	5.12	9.52	32,000	0.14	5.47	8.52	39,000	0.17	6.22	9.27	37,000
35	<0.01	3.08	8.77	-	<0.01	4.10	9.23	-	<0.01	4.25	8.54	-	<0.01	4.46	9.67	-
49	<0.01	1.52	6.63	-	<0.01	2.10	7.77	-	<0.01	2.63	7.71	-	<0.01	2.32	8.45	-
56	<0.01	0.74	5.82	-	<0.01	2.44	8.24	-	<0.01	2.11	6.32	-	<0.01	1.88	6.85	-

NA = not analyzed.

respectively. HCB concentrations decreased from approximately 5-6 µg/g to approximately 1-2 µg/g during the 28 days depuration study.

The results of the bioconcentration tests using DDE are presented in Table 3. The water concentrations in the DDE exposure also decreased about 15% during the exposures and the mean concentrations were all approximately 0.13 µg/l. DDE concentrations in the test fish were initially 0.10 µg/g and these residues increased about 9-fold to approximately 0.9 µg/g after two days of exposure. Like HCB exposures, the fish in the DDE tests continually accumulated DDE throughout the test and a steady-state condition was not observed. The C_F/C_w in Exposure 1 ranged from 6,200 after two days to 48,00 after 28 days. In Exposure 2, the C_F/C_w ranged from 6,600 after two days to 46,000 after 28 days. The range of two day and 28 day C_F/C_w in Exposures 3 and 4 were 7,700 to 57,000 and 5,500 to 50,000, respectively. DDE concentrations declined approximately 2 µg/g in the four tests during the depuration study.

The results of these tests are presented graphically in Figure 1. Figure 1(a) illustrates that the TCB exposure produced the only resemblence of steady-state for the three chemicals. Figures 1(b) and 1(c) clearly show that the 28 day bioconcentration factor underestimates the BCF for the bioaccumulative chemicals. In comparing the uptake curves in Figure 1, it must be recognized that the mean water concentrations in the TCB exposures were approximately 100 times those in the HCB and DDE exposures and that the absolute residue concentrations are not a measure of the bioaccumulation potential of the chemicals. Moreover, the apparent variability in concentrations in Figure 1 reflects variations in the water concentrations in addition to the analytical and biological variations.

TABLE 3. Summary of Analytical Results from p,p'-DDE Bioconcentration Tests

Test Day	Exposure 1				Exposure 2				Exposure 3				Exposure 4			
	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_E}{C_w}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_E}{C_w}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_E}{C_w}$	Water (µg/l)	Fish (µg/g)	Lipid (%)	$\frac{C_E}{C_w}$
0	0.15	0.10	6.06	-	0.13	0.10	6.06	0	0.12	0.10	6.06	-	0.13	0.10	6.06	-
2	0.15	0.94	7.99	6,200	0.13	0.86	7.20	6,600	0.12	0.93	7.91	7,700	0.13	0.72	8.02	5,500
4	0.14	1.19	7.55	8,500	0.18	1.44	8.27	8,000	0.12	1.45	8.81	12,000	0.19	1.28	8.59	6,700
8	0.13	2.39	NA	18,000	0.18	2.64	7.92	15,000	0.10	2.58	8.70	26,000	0.17	2.62	8.01	15,000
16	0.11	3.81	9.28	35,000	0.16	4.15	8.69	26,000	0.10	4.18	8.94	42,000	0.15	3.96	9.00	27,000
28	0.12	5.74	9.22	48,000	0.14	6.38	9.52	46,000	0.11	6.12	8.52	57,000	0.14	7.07	9.27	50,000
35	0.02	5.25	8.77	-	<0.01	5.70	9.23	-	0.02	5.69	8.54	-	<0.01	6.09	9.67	-
49	<0.01	NA	6.63	-	<0.01	5.40	7.77	-	<0.01	4.91	7.71	-	<0.01	5.70	8.45	-
56	<0.01	3.48	5.82	-	<0.01	6.05	8.24	-	<0.01	5.36	6.32	-	<0.01	5.10	6.85	-

NA = not analyzed.

The data are presented on a more comparative basis in Figure 2 in which the ratio C_F/C_w is plotted versus time of exposure. This plot corrects for the different exposure concentrations within the four tests with each chemical. Moreover, the relative accumulation between chemicals is apparent. This figure gives a visual summary of the precision of the exposures on a scale which should encompass most chemicals tested by this method.

Figure 3 presents the data from the depuration phase of this study. The data show that the DDE concentration in fish remained essentially constant during the 54 days in clean water. HCB was eliminated more rapidly than DDE while the residues of TCB declined rapidly to near the detection limit within one week. These data on elimination rates are inversely related to the BCF which is expected from the bioconcentration kinetics. If BCF at steady-state is the uptake rate divided by the depuration rate, chemicals with very small depuration rates should have large BCF if the uptake rate is comparable to other lipophilic chemicals.

DISCUSSION

This study demonstrates that the proposed method provides a reproducible test for measuring the bioconcentration factor. Using the 28 day BCF values for the four tests, the mean (± S.D.) BCF for TCB was 1,700 (±70) and the range was 1,600 to 1,800 in the four tests. The mean (± S.D.) BCF for HCB was 35,000 (±3,300) and the range was 32,000 to 39,000. The mean (± S.D.) BCF for DDE was 50,000 (±4,800) and the range was 46,000 to 56,000.

The greatest concern in estimating the BCF in the bioconcentration test is not the method of testing, but rather the method of calculating the BCF. As stated previously, the use of the 28 days BCF can only be an adequate measure of the bioaccumulation potential when the 28 day BCF is representative of steady-state residues. The 28-day BCF for HCB and DDE were clearly not at steady-state.

To compare different methods of estimating BCF from a given set of uptake and depuration data, the data were also analyzed using a modified BIOFAC computer program and a non-linear curve-fitting program, CANDLES, developed at ERL-D. Table 4 presents the results of the analyses. These results demonstrate that all three methods of estimating the BCF give essentially the same values for TCB, which can be expected since this chemical was tested to near steady-state. However, both the BIOFAC and CANDLES program estimated that the steady-state BCF for HCB and DDE are substantially higher than is estimated from the 28 day value. In the case of HCB, the BCF estimated from BIOFAC was 52,000 and that from CANDLES was 48,000. Compared to the 35,000 estimated from the 28 day ratio of C_F/C_w, the latter method is clearly inappropriate. For DDE, the BIOFAC method established a steady-state BCF of 180,000 while CANDLES estimated 110,00 compared to the 28 day value of 50,000.

TABLE 4. Comparison of BCF Values Computed by These Methods

	Estimated Bioconcentration Factor		
Method	TCB	HCB	DDE
ASTM, C_F/C_w at 28 days	1,700	35,000	50,000
BIOFAC	1,600	52,000	180,000
CANDLES	1,500	48,000	110,000

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LIST OF FIGURES

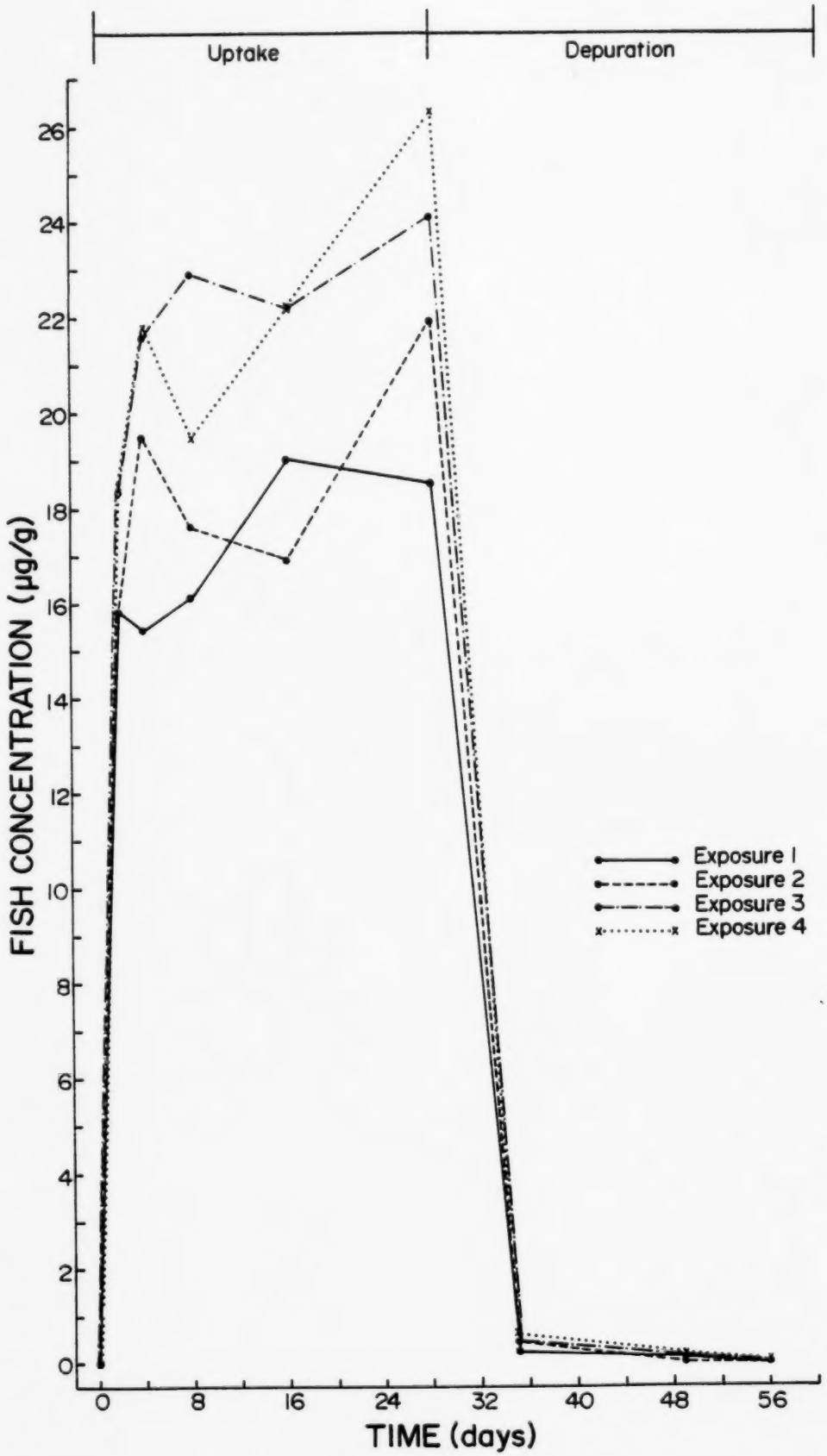
Figure 1(a) Uptake and depuration of 1,2,4-trichlorobenzene in fathead minnows (Pimephales promelas).

Figure 1(b) Uptake and depuration of hexachlorobenzene in fathead minnows (Pimephales promelas).

Figure 1(c) Uptake and depuration of p,p'DDE in fathead minnows (Pimephales promelas).

Figure 2 Uptake of TCB, HCB, and DDE in fathead minnows (Pimephales promelas).

Figure 3 Depuration of TCB, HCB, and DDE in fathead minnows (Pimephales promelas).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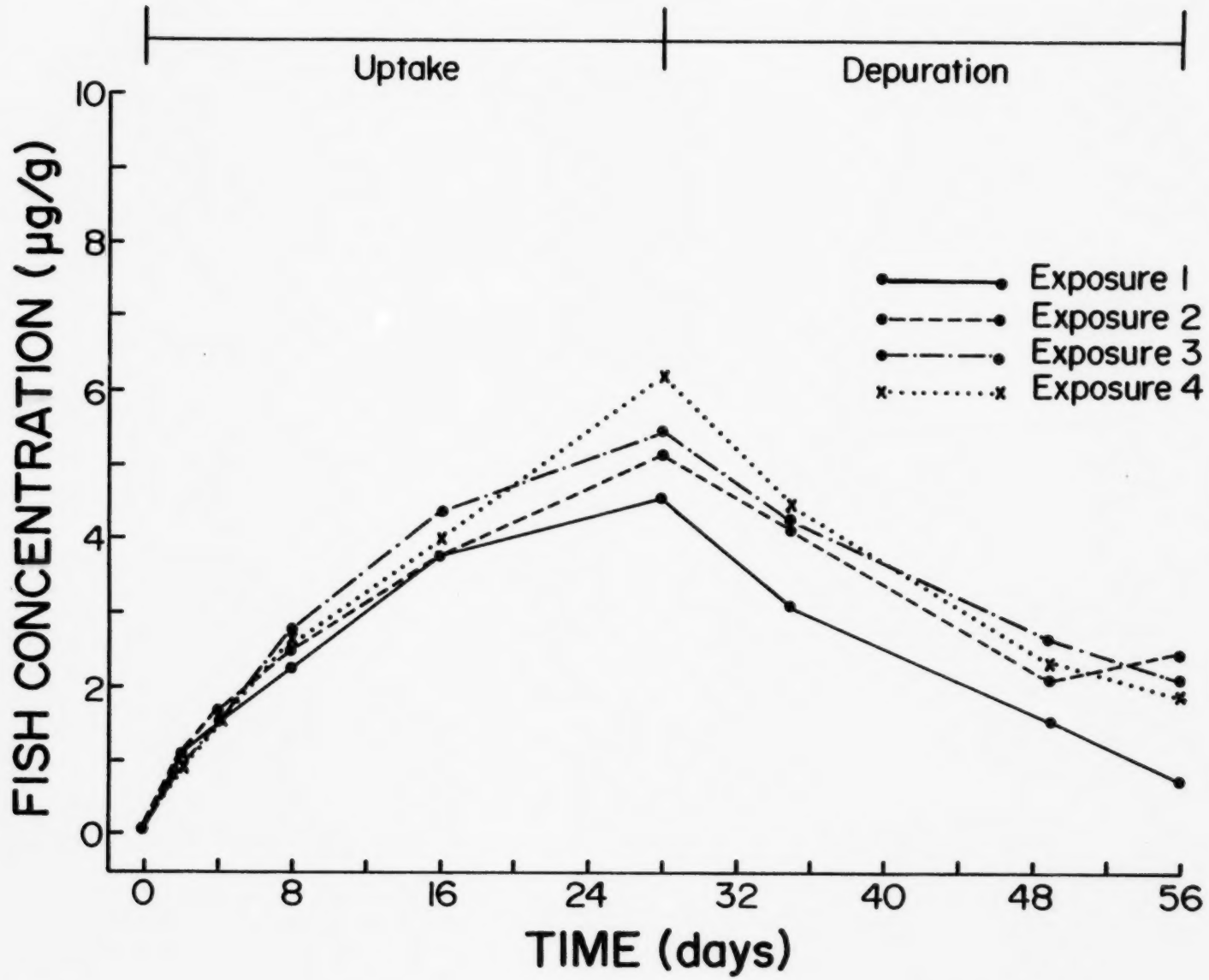
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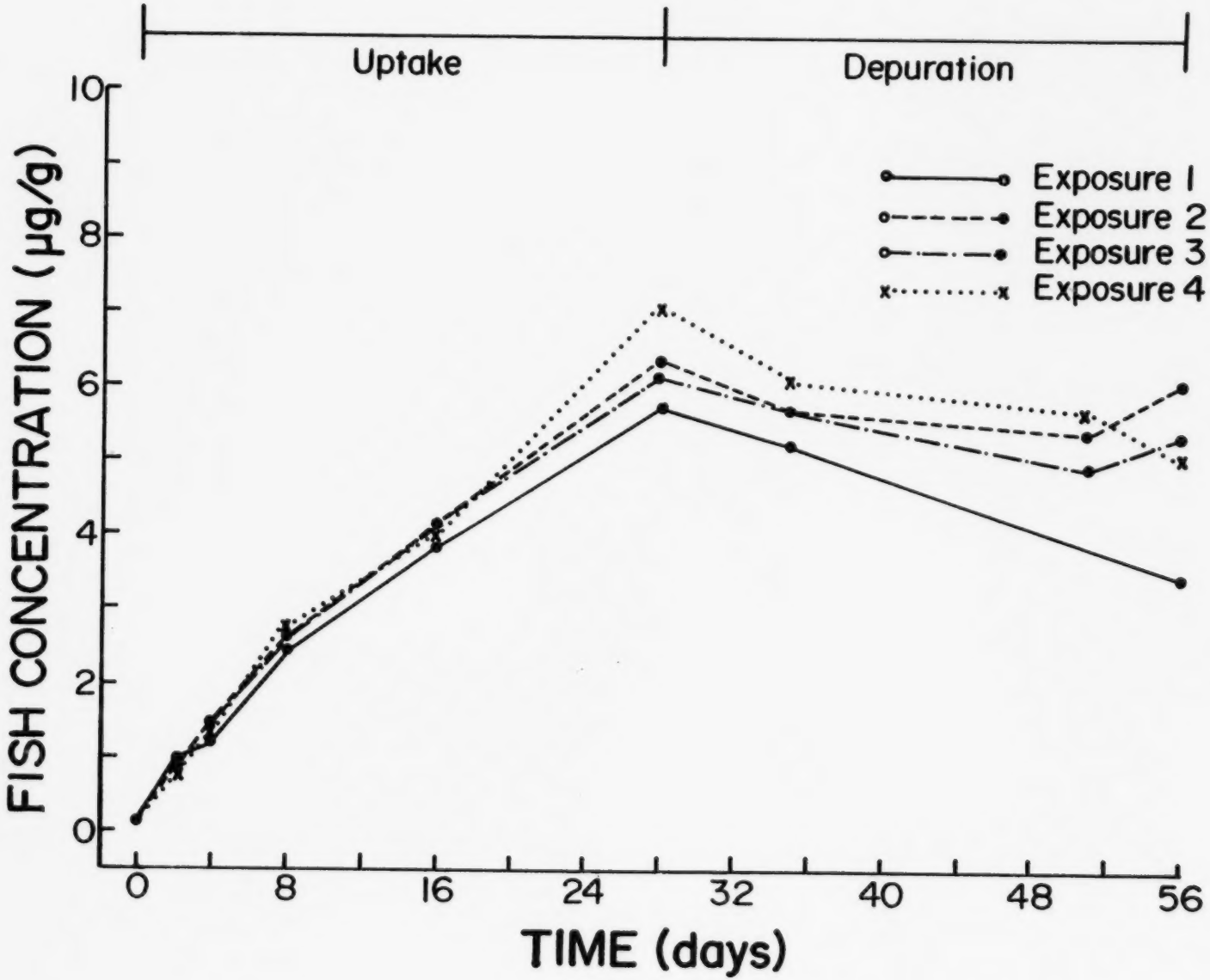
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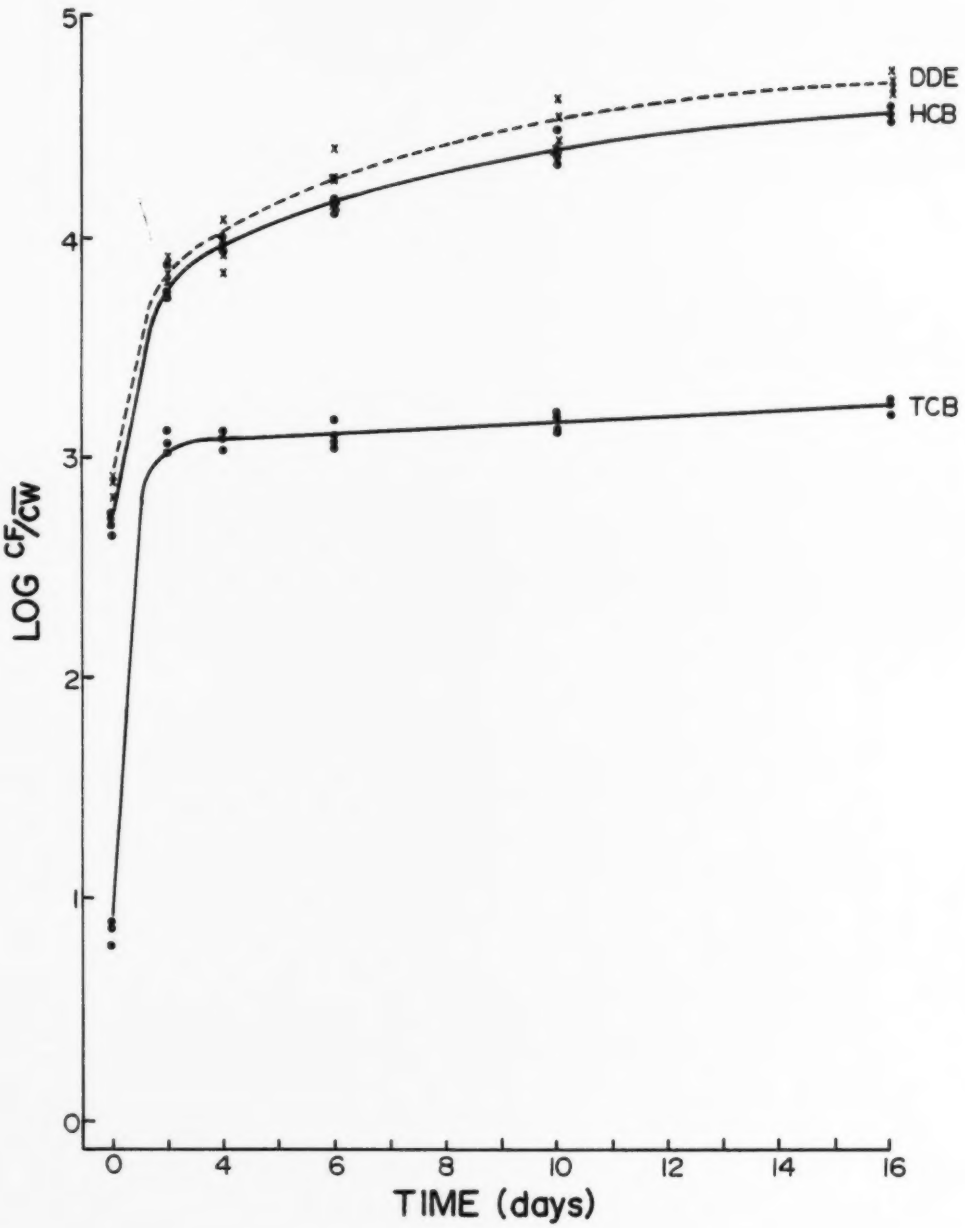
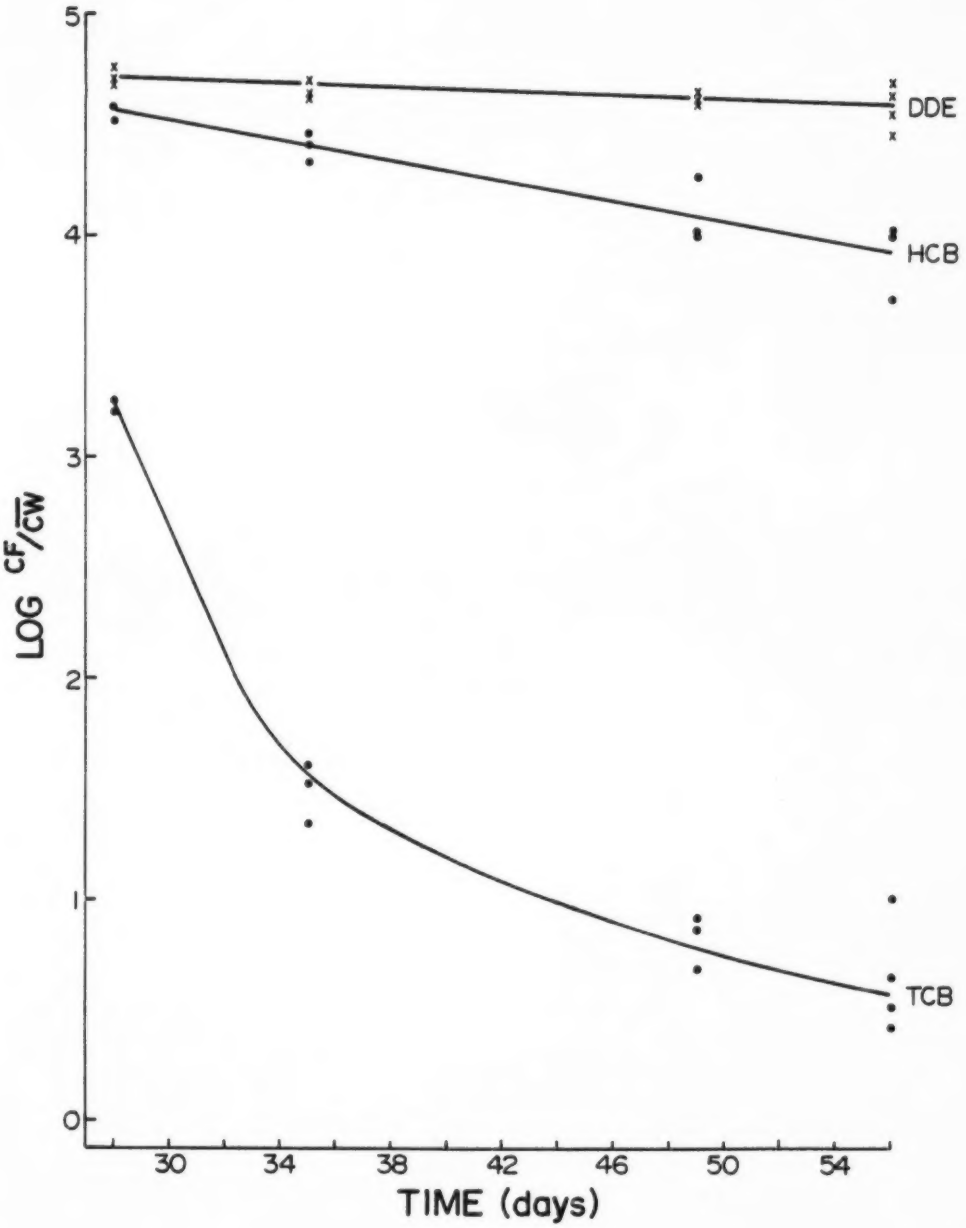
56
TIME (days)


5
4
3
2
1
0
LOG $C_F/\overline{C_W}$
DDE
HCB
TCB
0
4
6
8
10
12
14
16
TIME (days)


5
4
3
2
1
0
LOG $\frac{CF}{CW}$
DDE
HCB
TCB
30
34
38
42
46
50
54
TIME (days)